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Perspective

Diseases of Bone and the Stromal Cell Lineage

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INTRODUCTION

FOR DECADES, the quest for a more complete insight into mechanisms of bone disease has promoted the development of in vitro systems that model the two main cell types responsible for bone formation and remodeling, osteoblasts and osteoclasts. While generating an unprecedented explosion of biological information, osteoblastic models have been both diverse and controversial. Since the 1980s, a reappraisal of earlier studies on the biology of bone marrow stromal cells as related to the origin and differentiation of osteoblasts have led to ever-increasing current attention to their use and value in bone biology and disease. Significant advances in the pathophysiology of osteoporosis, for example, were enabled by this shift in focus from purportedly ''osteoblastic'' models to marrow stromal cells proper.[(1)]

Stromal cells are amenable to, and can be assayed by, in vivo transplantation for the direct assessment of bone tissue formation and organization at the histologic level. Furthermore, they form histologically proven bone, a dimension long missed in the evaluation of osteoblastic differentiation and function in in vitro models. They impose consideration of the dynamics of cell birth, differentiation, and loss. However, because they do not represent a direct ex vivo counterpart of the ''osteoblast-osteoclast'' cliché (which imprints the format of all bone meetings), they force the inclusion of other elusive (or eluded) cell types found within the bone/bone marrow environment into our understanding of cellular players of bone physiology and disease. In this remodeled view, pathological changes in bone attain at the same time a cell lineage and an organ dimension, instead of the tissue dimension characteristic of histomorphometric readings of bone pathology, which traditionally have arrested at the untrespassed Hercules pillars provided by the bone-lining cells. Consequently, marrow stromal cells are more than simply an alternative strategy. When adopted as tools, they convey an inherently different angle for the investigation of the biology of bone disease.

BONE MARROW STROMAL CELLS AND THEIR TRANSPLANTATION ASSAYS

The ability of bone marrow cells to recapitulate the formation of a miniature bone organ (that is, a complex of bone and hematopoietic bone marrow with appropriate mutual architectural layout) was first demonstrated by Friedenstein and coworkers in the late 1960s by transplanting small pieces of intact marrow (devoid of bone per se) underneath the kidney capsule in mice.[(2,3)] Using in vitro analysis of single-cell suspensions of bone marrow, Friedenstein identified an adherent, nonhematopoietic stromal cell capable of giving rise to discrete colonies (clonogenic), the colony forming unit-fibroblast (CFU-F).[(4,5)] Friedenstein and his colleagues went on to prove that bone marrow stromal cells are, in fact, responsible for the generation of bone and bone marrow by repeating the kidney capsule transplantation using this ex vivo expanded population of cells. Using chromosomal markers, it was determined that not only bone but also hematopoiesis-supportive stroma and associated adipocytes were of donor origin, while hematopoiesis was of recipient origin in Friedenstein's ''open'' transplants, which allow for access of host blood-borne cells to the site of transplantation.[(4,6–8)] Subsequently, Owen and others confirmed the ability of the stromal cell population to give rise to the same spectrum of connective tissue phenotypes (bone, reticular fibrous tissue, adipocytes, and also cartilage) in a closed system, as dictated by the use of diffusion chambers, disallowing direct contact of the cells with recipient tissues but allowing nutrient transfer, and in the absence of hematopoiesis.[(9–14)] More recently, open systems whereby ex vivo expanded stromal cells from any animal species can be transplanted into the subcutis of immu-

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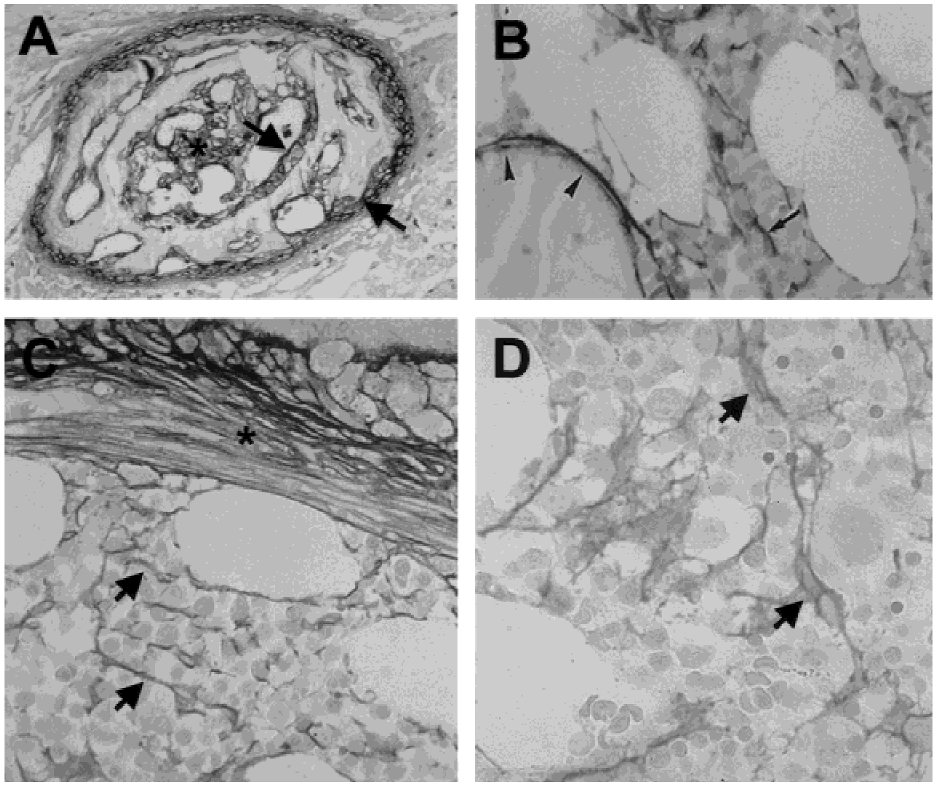

FIG. 1. (A) Cross-section of a developing rib of a rat fetus, stained for ALP. Osteogenic tissue is highlighted around and inside (arrows) the developing rudiment. The ALP-reactive tissue in the developing marrow cavity (*) represents the primitive stroma of the bone marrow, which is not yet populated by hematopoietic cells. (B) Human iliac crest bone biopsy, ALP staining. ALP activity highlights the thin processes of ''reticular'' (Westen-Bainton) cells within the marrow, which would not be detectable by conventional histology (arrow). Arrowheads point to ALP-positive bone-lining cells. (C and D) Human iliac crest bone biopsy from a patient with secondary hyperparathyroidism, ALP staining. Note the dramatic increase in elongated, fibroblast-like, ALP-positive cells along the bone surface (so-called ''endosteal fibrosis'') (*), and the increased numbers of ALP-positive cells in the marrow stroma proper (arrows).

nocompromised mice have been further developed and refined.[15–19] The ability of individual CFU-F to regenerate the complete spectrum of phenotypes found in Friedenstein's original system—that is, osteogenic, hematopoietic supporting, and adipose cells—was demonstrated recently using these novel approaches.[19]

The open transplants of marrow-derived stromal cells demonstrated the potential of these cells to generate not only histology proven, authentic bone tissue, but also a hematopoietic microenvironment. The two events are inextricably interconnected. One cannot establish an ectopic hematopoietic microenvironment without establishing ectopic bone first. There is compelling developmental evidence for this. In development, bone formation always precedes the establishment of a bone marrow. Bone marrow stroma is initially established as an osteogenic tissue itself, only to be later colonized by blood-borne hematopoietic stem cells upon the development of an appropriate sinusoid system[20,21] (Fig. 1A). Accordingly, formation of the marrow lies downstream of the formation of bone tissue, but as part of a single, strictly coordinated program for organogenesis that evolves from cells derived from an osteogenic tissue and represents a local adaptation of an otherwise osteogenic embryonic tissue.

This sequence of events is faithfully recapitulated in the development of ectopic ossicles subsequent to transplantation of postnatal stromal cells[22,23] (Shi, Kuznetsov, Bianco, and Gehron Robey, unpublished data). Bone is established first and represents a *conditio sine qua non* or the later appearance of (host) hematopoiesis in a (donor) stromal scaffold. Not only does the ossicle that is formed upon transplantation of ''stromal'' cells mimic the structure of a bone organ, it recapitulates the correct developmental temporal pattern.

BONE MARROW PREOSTEOBLASTS

If indeed bone-forming cells and cells in the bone marrow stroma belong to a lineage (much like red blood cells and erythroblasts belong to one hematopoietic lineage), then one would expect that the lineage unfolds, in vivo, through defined steps corresponding to recognizable cell phenotypes. Unfortunately, only two stages are generally recognized in the osteoblast lineage which are defined by criteria of different natures: an in vitro assay for CFU-F and a histologic dimension and identity for mature osteoblasts, which can only be defined as a basically nonproliferative cell in the process of making bone matrix proper. Our understanding of the stages that lie in between is limited. On a histologic scale, we can easily identify the immediate precursors of the mature osteoblasts (preosteoblasts) associated with the developing periosteal bone surfaces.[21,24] While one would assume that preosteoblasts would be easily identified at any other site of bone deposition during development, growth, and remodeling, recognition of an equivalent cell phenotype within the intact bone marrow (relevant for example to adult bone remodeling) has lagged behind. Observations on the developing and postnatal marrow of humans and rodents point to cells associated with the outer surface of marrow sinusoids.[21,25] These cells (the so-called Westen-Bainton cells[26]) (Fig. 1B) noted for a reticular morphology, strong alkaline phosphatase (ALP) activity are likely the marrow equivalents of periosteal preosteoblasts. The same cells represent direct precursors of marrow adipocytes in vivo.[27]

ABNORMAL MARROW IN BONE DISEASES

As part of a single organ, bone and the bone marrow stroma react to disease in a coordinated fashion resulting in detectable changes in both bone tissue proper and the marrow stroma.[28] General homeostatic mechanisms controlling the size and balance of different cell compartments (precursor cells and mature effector cells) within a dynamic lineage must also apply to the osteoblast lineage, as is the case with hematopoietic lineages (with which analogies are often drawn to conceptualize the stromal/osteoblastic lineage). Expansion or shrinkage of immediate blood cell precursor compartments (hyperplasia or hypoplasia) accompanies, for example, disorders of the blood cells. In principle, therefore, immediate precursors of mature osteoblasts must also come into play in regulating the balance of bone tissue mass and turnover. A number of events or adaptive responses must be regulated, in bone as in any other tissue featuring reserve cells, downstream of the ultimate ''stem'' cell compartment. These events should be reflected in detectable changes in size and organization of the compartment of marrow preosteoblasts in bone disease. Changes of this kind do in fact occur (Figs. 1C and 1D) Histologically, in several bone disorders, altered numbers and distribution of ALP positive cells in the marrow spaces are sometimes at least as prominent as the changes in the number, architecture, or quality of bony structures themselves.[29,30] Nonetheless, as a reflection of the limited awareness of the identity and characteristics of the marrow preosteoblasts, these changes and their significance with respect to the abnormalities in bone tissue proper are poorly appreciated and often dismissed with descriptive and inaccurate definition of their nature.

THE PARADIGM OF FIBROUS DYSPLASIA

Fibrous dysplasia of bone (FD; here taken as part of the McCune–Albright syndrome, but also in most cases in which it presents as an isolated disorder) represents an interesting paradigm of how bone and marrow interplay in disease. The paradigm of FD also highlights how a prominent disease of bone as an organ can remain unrecognized as a disease of cells in the osteoblast lineage, even once its underlying genetic defect is identified. In FD, obvious changes in structure and organization occur both in bone and marrow. Together, the bone and marrow changes observed in FD result in serious focal skeletal lesions, with severe consequences on the mechanical performance and structural integrity of individual skeletal segments. Bone is focally abnormal in structure and amount, and a tissue usually described as ''fibrotic'' fills the spaces between the abnormal trabeculae. Textbooks of bone pathology read almost invariably that FD is the overgrowth of fibrous tissue within which abnormal bone is formed with no intervention of bone-forming cells (an obvious paradox). Recent studies have shown, in contrast, that FD bone is abnormal in matrix composition and collagen organization as a result of malfunction of mature osteoblasts.[30,31] These studies also showed that the ''fibrotic'' tissue itself differs from common fibrosis found in other organs and diseases. Far from being a nondescript scar-like tissue, it was characterized as an excessive accumulation of cells whose phenotype closely mimics the one associated with histologically defined preosteogenic cells in the normal marrow and in other normal osteogenic tissues. Interestingly, similar changes are observed in human hyperparathyroidism, and curiously, they are also described, commonly, as marrow/endosteal ''fibrosis.''[29] FD of bone and hyperparathyroidism share several similarities as far as the nature of bone lesions is concerned. They are also closely related pathogenetically. While bone lesions in hyperparathyroidism are generated by excess PTH, in FD the signal transduction pathway that is a major player in PTH signaling is constituitively overactive due to a missense mutation in the α subunit of the stimulatory G protein, Gs.[32–34] Thus, downstream effects of excess ligand binding or ligand-independent receptor activity lead to similar effects on the target cells, which in this case, are osteogenic.

FD can thus be seen as a disease resulting from a generalized derangement in the biology of the osteogenic lineage in the bone and marrow environment, which translates into defined, recognizable histologic changes both in the marrow stromal preosteoblast compartment and in the mature bone-forming cells. In other words, it is an osteogenic cell dysplasia.

TRANSPLANTATION OF DISEASED STROMAL CELLS

If transplanted marrow stromal cells are able to recapitulate and establish ectopically the development of normal bone and normal marrow in the appropriate sequence, one can envision that genetically abnormal stromal cells would dictate, likewise, the development of an abnormal bone and marrow. One can then exploit the logic of the transplant system to probe not only the ability of stromal cells to establish and maintain a physiology, but also to convey and

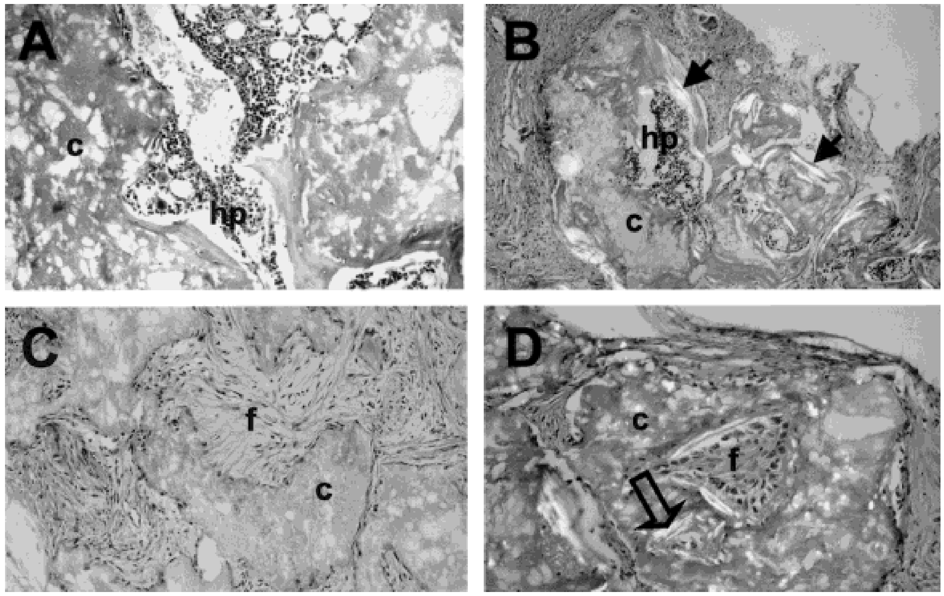

FIG. 2. Ectopic ossicles formed in immunocompromised mice following transplantation of multicolony-derived strains of marrow stromal cells with ceramic particles (c) from normal donors (A and B) or FD patients (C and D). Note the development of hematopoietic tissue (hp), adipocytes (a), and abundant lamellar bone (arrows) in normal ossicles, and the absence of hematopoiesis and adipogenesis in the MAS ossicle, in which fibrotic tissue (f) and limited amounts of woven bone (open arrow) are formed, mirroring the histology of native fibrous dysplastic lesions.

reproduce a disease of bone as an organ. Again, FD provides an ideal model. Not only because it features interconnected alterations in bone and marrow that are histologically obvious, but also because the activating mutation causing the disease has been identified[32,33] and demonstrated in the CFU-F isolated from patients' marrow.[35] Marrow stromal cells isolated from fibrous dysplastic bone and transplanted into immunodeficient mice indeed result in the establishment of an abnormal FD-like ossicle. At variance with their normal counterparts (Figs. 2A and 2B), marrow stromal populations containing Gsα-mutated cells generate bone tissue that is inadequate both quantitatively and qualitatively (Figs. 2C and 2D) as evidenced by their inability to establish a hematopoietic microenvironment and feed into the adipogenesis pathway.

This pilot exercise, transplantation of diseased stromal cells, highlighted important, previously unrecognized aspects of the biology of FD (for example, the essential role of somatic mosaicism in the development of a FD lesion, that is, that a mixture of both normal and mutant cells are required to regenerate a fibrous dysplastic ossicle). Thus, it taught something about the disease itself. In more general terms, it proved the principle that diseases of bone as an organ, featuring histologically detectable changes both in the bone and marrow tissues, can be reproduced upon in vivo transplantation of precursor cells of the stromal system, at least in the circumstance in which a genetic defect is involved. Transplantation of mutated stromal cells results in a ''transfer of disease'' to the ectopic ossicle that develops in the transplantation site.

At a glance, it may at first appear self-evident that a gene defect in ''stem'' cells would express its impact on the whole progeny of a putative stromal stem cell, if such a cell exists. However, the value of a transplantation assay, as manipulated to the investigation of diseases rather than normal development, rests specifically with probing when and where in the osteoblast lineage a given gene defect has its impact in the establishment of a normal bone/marrow unit, and what goes wrong as a result. One would predict that different mutations would affect different facets of the program for bone organogenesis that is recapitulated in the transplantation system, thus illuminating the underlying target events. As applied to FD, the transplantation assay proved that activating mutations of the GNAS1 gene have a critical negative impact on the establishment of the hematopoietic microenvironment and on the development of marrow adipocytes, thus indicating the significance of normal Gsα-mediated signaling for hematopoiesis–stroma interactions.

The dialogue between in vitro reductionistic models and in vivo models has been seen as a sorely needed scenario of bone biology over the past 20 years. The diversity of the cell culture models and the lack of authentic bone tissue formation in most models (and of bone organ formation in all models) have hampered the achievement of what should be a standard mode of investigation. Perhaps the in vivo culture compromise offers a more direct access. Obviously, assessing not only the osteogenic differentiation potential, but also the osteogenic ''performance'' (quantity and quality of authentic bone tissue that forms) by means of the most direct evidence provided by the histologic dimension provides an appealing advance. We believe that the value of this advance will prove itself specifically in the area of ''osteogenic lineage diseases.'' By this term, we mean intrinsic defects in the cellular machinery of cells in the osteoblastic lineage, taken to encompass CFU-Fs to the mature, competent osteoblast via the committed cells found in the bone marrow stroma in vivo. This term also includes the amenability of these changes to be assessed by an open transplantation system of stromal cells in immunocompromised mice that is conducive for bone cell differentiation. We predict that transplantation of stromal cells carrying different genetic defects will result in miniature bone organs with different structures of bone and marrow tissues. Standardization of transplantation conditions and refinement of the evaluation of the outcome of the procedure are, of course, necessary steps to be completed before this approach will unfold to its full potential. Yet, even as is, important advances have already been made. Experience with transplants of either multicolony derived strains or single colony–derived strains under reproducible conditions is starting to delineate a "transplant physiology" (timing, quality, and quantities of tissue formation) against which the behavior of abnormal transplants can be compared. Single cells in the tissues resulting from transplantation can be recognized easily as of donor versus host origin using probes for species-specific DNA sequences in tissue sections. The amount of bone tissue can be quantitated, and cells can be characterized with the full range of molecular probes and techniques available today. Finally, the competence of donor stroma to fully support host hematopoiesis can be evaluated.

When used to explore the consequences of natural mutation, the generation of living human "miniature" diseased skeletal organs would provide obvious advantages in the quest for novel and appropriate therapeutic strategies, including genetic engineering of mutated cells. Furthermore, these in vivo cellular models may complement or at times substitute for alternative approaches, such as the development of transgenic animals. Generation of an ectopic bone–marrow organ is independent of development of other associated tissues, such as cartilage, tendon, muscle, etc., which often influences indirectly bone physiology (e.g., fibroblast growth factor receptor mutations that affect primarily cartilage development, but may also affect, albeit less dramatically, bone formation as well). Likewise, use of this approach for investigating the effects of targeted mutations should prove to be of value in dissecting their specific impact on the osteogenic lineage.

Bone diseases that have captured most attention to date can often be seen as localized effects on the skeleton of mechanisms that are extrinsic to it. The established notion of a marrow stromal lineage which includes osteogenic cells reminds us that in other diseases, intrinsic changes in the biology of cells must impact at various stages in that lineage, and that these changes are reflected by alterations of multiple tissues within the bone organ. The conceptual and experimental tools are now at hand for starting to tackle them in a rational way.

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